5' CCAGCAGCGACTCTGAGG (upstream primer; SEQ ID No. 13)

5' CCAAGACGTTGTGTGTTC (downstream primer; SEQ ID No. 14)

It is further understood that other primers for qualitative or quantitative amplification of c-myc cDNA or mRNA are suitable for use as designed using methods known to the art, for example but not limitation primers described by Gamberi *et al.* (1998, *Oncology* 55: 556-563), Sagawa *et al.* (2001, *Cancer Letters* 168: 45-50), Christoph *et al.* (1999, *Int. J. Cancer* 84: 169-173), and Latil *et al.* (2000, *Int. J. Cancer* 89: 172-176), these references incorporated herein by reference in their entirety.

For hnRNP A2/B1 RNA RT-PCR, the preferred primers are those described by Zhou *et al.* (1996, *J. Biol. Chem.* 271: 10760-10766), herein incorporated by reference in its entirety, wherein primers for PCR of hnRNP A2/B1 associated cDNA have the sequence

5' GAGTCCGGTTCGTGTTCGTC (SEQ ID No. 15)

5' TGGCAGCATCAACCTCAGC (SEQ ID No. 16)

It is further understood that other primers for qualitative or quantitative amplification of hnRNP A2/B1 cDNA or RNA, or for amplification of associated RNA such as hnRNP A2 RNA or cDNA and hnRNP B1 RNA or cDNA, are suitable for use as designed using methods known to the art.

In one example of a preferred embodiment, RNA is harvested from approximately 1.75 mL aliquots of serum or plasma, and RNA extracted therefrom by the Perfect RNA Total RNA Isolation Kit (Five Prime – Three Prime, Inc., Boulder, Colorado) according to manufacturer's

instructions. From this extracted RNA preparation, 10 - 20 microliters are reverse transcribed to cDNA as described above.

In a preferred embodiment, RT-PCR for EGF mRNA is performed by the method of Rajagopal *et al.* (1995, *Int. J. Cancer* 62: 661-667), incorporated herein by reference in its entirety, using 19 microliters of the EGF cDNA in a final volume of 100 microliters in a reaction mixture containing 2.5U of AmpliTaq DNA Polymerase (Perkin Elmer Corp., Foster City, California), 80 microliters of PCR buffer containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 400 microM each dNTP, and 0.125 microM each of Primer SEQ ID No. 1 and Primer SEQ ID No. 2 identified above. The mixture is amplified in a single-stage reaction in a thermocycler under a temperature profile consisting of an initial 5 minute incubation at 94 degrees C, followed by 40 cycles of denaturation at 94 degrees C for 10 seconds, annealing at 63 degrees C for 30 seconds, and extension at 72 degrees C for 30 seconds, followed by a final extension at 72 degrees C for 10 minutes. Detection of the amplified product is achieved, for example by gel electrophoresis through a 3% Tris-borate-EDTA (TBE) agarose gel, using ethidium bromide staining for visualization and identification of the product fragment.

In alternative preferred embodiments, qualitative or quantitative amplification for EGF mRNA is performed by other methods known to the art, *for example*, methods as described by Dahiya *et al.* (1996, *Urology* 48: 963-970); LeRiche *et al.* (1996, *J. Clin. Endocrinol. Metab.* 81: 656-662); or Pfeiffer *et al.* (1997, *Int. J. Cancer* 72: 581-586), wherein these references are incorporated by reference herein in their entirety.

In a preferred embodiment, PCR amplification of EGFr cDNA is performed by the method of De Luca *et al.* (2000, *Clin. Cancer Res.* 6: 1439-1444), herein incorporated by reference in its entirety. Eight microL of EGFr cDNA is used in a 25 microL reaction buffer

containing 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 0.25 mM each dNTPs, 0.5 U Taq Gold polymerase (Perkin-Elmer), and 10 picomoles each of Primer A (SEQ ID No. 5, identified above) and Primer B (SEQ ID No. 6, identified above). The mixture is amplified in a two stage reaction in a thermocycler. In the first stage reaction, PCR is performed for 30 cycles under a temperature profile consisting of an initial 10 minute incubation at 94 degrees C, followed by 5 cycles of denaturation at 94 degrees C for 30 seconds, annealing at 60 degrees C for 45 seconds, and extension at 72 degrees C for 45 seconds, followed by 25 cycles of denaturation at 94 degrees C for 30 seconds, annealing at 55 degrees C for 45 seconds, and extension at 72 degrees C for 45 seconds, with the extension lengthened to 10 minutes during the last cycle. One microliter of the first stage product is then used for the second stage nested PCR in a mixture prepared as in the first stage except that the primers used are now Primer A (SEQ ID No. 5, identified above) and Primer C (SEQ ID No. 7, identified above). In the second stage reaction, nested PCR is performed for 35 cycles under a temperature profile consisting of an initial 10 minute incubation at 94 degrees C, followed by 5 cycles of denaturation at 94 degrees C for 30 seconds, annealing at 60 degrees C for 45 seconds, and extension at 72 degrees C for 45 seconds, followed by 30 cycles of denaturation at 94 degrees C for 30 seconds, annealing at 55 degrees C for 45 seconds, and extension at 72 degrees C for 45 seconds, with the extension lengthened to 10 minutes during the last cycle. The amplified product can then detected by gel electrophoresis through a 1.5% agarose gel with visualization by ethidium bromide staining. The amplified product can further be hybridized to an EGFr cDNA probe and visualized for example using streptavidin-alkaline phosphatase-coupled enhanced chemiluminescence (New England Biolabs, Beverly, Massachusetts).